CAROTENE AND VITAMIN A : THE ANTI-INFECTIVE ACTION OF CAROTENE.

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RATS fed on a diet devoid of vitamin A but complete, so far as is known, in other dietetic units ultimately develop multiple infective lesions and die (Green and Mellanby, 1928). We have previously dealt with this phenomenon, and also attempted to see whether the experimental observation in animals had any clinical significance by giving large amounts of vitamin A for therapeutic purposes to a small number of patients suffering from puerperal septicæmia (Mellanby and Green, 1929). In this report we wish to deal with a specific problem, namely, as to whether carotene has the characteristic anti-infective action previously shown to be possessed by vitamin A.

The prolonged discussion on the relation between the carotenoid pigments and vitamin A has now been placed on a more definite footing by the work of Euler, Euler and Hellström (1928), who have shown that samples of crystalline carotene, presumably pure, possess growth-promoting properties similar to those of vitamin A. Many other carotenoid pigments were tested, but only one, α-dihydrocrocetin, was found to be active (Euler, Euler and Karrer, 1929).

The literature of the sequence of events which at one time and another has led to a denial of this relationship is surveyed in two recent papers—Collinson, Hume, Smedley-Maclean and Smith (1929), and Moore (1929)—and therefore need not be further discussed here. The present position, however, is still a little obscure since, on the one hand, Dulière, Morton and Drummond (1929), using a sample of carotene of a high degree of purity, found it inactive. At present it is suggested that their use of a fat-free basal diet may explain the discrepancy, for it is possible that carotene is only absorbed in the presence of fat. On the other hand, the work of Collinson *et al.* (1929) and Moore (1929) indicates that increasingly pure samples of carotene do not possess, at any rate, less " vitamin A " activity.

It is clear that, if carotene has the properties usually associated with vitamin A, and even if carotene is the actual compound responsible for the activity of substances such as green and other vegetables, butter and egg yolk, as it might well be, it is not the same substance as vitamin A in liver fats. The carotene content of cod or mammalian liver fat can only at the best be very small, yet preparations from these substances can be obtained which have as much or even more vitamin A activity than crystalline carotene. Liver oils containing vitamin A give with $SbCl_3$ a transient blue colour with an absorption

band at 610–630 $\mu\mu$, whilst carotene gives a permanent blue colour with the same reagent with an absorption band at 590 $\mu\mu$. Again, vitamin A in liver oils can be readily extracted from the unsaponifiable fraction by alcohol, whilst carotene is only slightly soluble in alcohol. It is possible that the association of vitamin A with other lipoid material when prepared from liver oils may modify some of its attributes, but the weight of evidence is strongly in favour of its being a substance other than carotene.

Most of the experimental work on carotene and its relation to vitamin A has depended on observations concerning its effect on the weight of rats which have begun to lose weight as the result of vitamin A deprival. Our observations on the action of vitamin A in raising the resistance of the body to auto-infection seemed so important that we felt it desirable to test the action of carotene in its relation to infection. Obviously if carotene has the biological properties of vitamin A it should be equally effective in preventing the occurrence of this generalized infection. Preliminary experiments quickly indicated that it was probably as efficient as vitamin A in this respect, and further work was only required to determine the minimal doses which had complete protective and curative effects.

METHODS AND RESULTS.

The feeding technique was in all essentials that previously described by us (1928). The basal diet devoid only of vitamin A contains a considerable quantity of fat chiefly in the form of heated olive oil, and the calcium-phosphorus ratio is a good one.

The animals used were albino rats bred from stock rats which have been reared under standard conditions for several years.

Vitamin D was given in every case, with the exception noted in the tables, each rat receiving $\frac{1}{2}$ drop of radiostol (irradiated ergosterol) daily.

The sample of crystalline carotene used had a melting-point of 174° (in air), and the same specimen was used throughout. It was dissolved in freshly distilled ether. The required volume of solution was allowed to evaporate on a little casein, which was then intimately mixed with a little of the basal diet. The "bit" so made was consumed by the rat before any more food was given. Control rats received an equivalent amount of extra casein. The ethereal solutions of carotene were prepared weekly and stored in the refrigerator.

The experimental findings as set out in tabular form are in the main self-explanatory. It will be noticed that in Experiment I there was no preliminary depletion period on a —A diet; that is to say, the experiment was started at a time when there was still a supply of vitamin A in the livers and other organs of the animals. Some of these animals were fed on diets containing varying quantities of carotene and others were used as controls and given the —A diet. In Experiments II and III, a preliminary depletion period on a —A diet of 32 and 52 days' duration respectively was introduced. During these preliminary periods the animals were thus deprived of their vitamin A stores, and many of them in Experiment III developed infective foci and lost weight. In this experiment the supplementary carotene had therefore often to bring about partial or complete recovery from the infected state before resumption

of growth and improvement in health could be effected. Thus in Experiment I the test consisted essentially of determining the prophylactic, and in Experiment III the curative action of carotene.

DISCUSSION OF RESULTS.

It is obvious from the above results that carotene acts as a very potent anti-infective agent. From Tables I–IV it will be seen that the response to carotene is as reasonably quantitative as could be expected. Complete protection against infection was always found when greater amounts than 0·04 mgm. of carotene were given daily, even when a prolonged depletion period on a — vitamin A diet had preceded the addition of the carotene. Many of these animals must have developed an infection in the depletion period (on the basis of previous results) and yet complete recovery and good health followed. In the case of one animal in Experiment III receiving 0·04 mgm. of carotene no recovery followed and the animal died on the 107th day. This is an exceptional result, for even when only 0·02 mgm. of carotene was given to 3 animals in the same experiment they remained healthy and continued to grow throughout the feeding period. In the case of the animals receiving only 0·01 mgm. of carotene the protection against infection was less certain, and some died with organs invaded by micro-organisms. With 0·005 mgm. of carotene the protection afforded was still less, although even in this case, when the animals are compared with the controls receiving no carotene, there is evidence of an increased resistance. It is difficult to state the exact protective dose of carotene in the case of the rat, but probably in the average animal when no running-out period (*i. e.* deprival of vitamin A) is allowed, a daily dose of 0·02 mgm. is sufficient. After the animal's body has been completely deprived of vitamin A and infective foci have probably started prior to the addition of carotene, a dose of 0·02 mgm. seems to be enough to restore perfect health and

TABLE I.—*The Comparative Effects of Varying Quantities of Carotene on Resistance to Infection. Exp. I: No Preliminary Depletion Period.*

Duration of experiment, 119 days.

Nature of supplement.	Survival time.	Site and intensity of infection.	Growth response (in gm.).	Remarks.
0·01 mgm. carotene	To end of experiment	M.E.	35–122	General condition good.
Ditto	Ditto	M.E.; mastoid	34–140–131	,, ,,
,,	,,	M.E.	40–130	,, ,,
0·005 mgm. carotene	,,	T.A.++	35–119–97	Scanty abdominal fat.
Ditto	,,	T.A.++	39–124–111	,, ,,
,,	106	X.+; T.A.+; M.E.+; bladder stone	36–96–76	No ,,
Nil	50	T.A.+++; enteritis; uterus (cornu); X.+	47–119–106	...
,,	39	X.+; T.A.+++; N.S.; bladder; kidney	42–129–121	...
,,	59	T.A.+++; M.E.++	39–101–84	...

T.A. = Tongue abscess. X. = Xerophthalmia. N.S. = Nasal sinus. M.E. = Middle ear.

TABLE II.—*Exp. II: Partial Preliminary Depletion Period. (32 Days on —A Basal Diet prior to adding Supplement.)*

Duration of experiment, 125 days.

Nature of supplement after partial depletion period.	Survival time.	Site and intensity of infection.	Growth response (in gm.).	Remarks.
0·005 mgm. carotene	To end of experiment	T.A.+++; colon	115–142–122	Scanty abdominal fat thymus very small.
Ditto	Ditto	X.+; T.A.+++	125–150–136	Ditto.
,,	,,	T.A.+	106–134–95	Thymus fair size; fair amount abdominal fat.
,,	107	T.A.±; mastoid; N.S.; bladder	94–122–86	Bladder hypertrophied, with shaggy inflamed mucous membrane; left ureter greatly dilated; pelvis of left kidney dilated and full of infected necrotic material.
0·01 mgm. carotene	42	...	105–90	Cause of death unknown.
Ditto	To end of experiment	T.A.±	120–162	Normal thymus; fair amount abdominal fat.
,,	70	X.+; M.E.++	110–100	...
,,	64	Enteritis	85–90–75	...
0·01 mgm. carotene (No vitamin D)	77	Cæcum and colon; bladder	97–124–86	Litter 14 days previous to death; hæmaturia prior to death.
Ditto	To end of experiment	...	108–140	Normal thymus; fair amount abdominal fat.
,,	Ditto	...	105–139–133	Healthy.
,,	,,	...	96–148	,,
0·02 mgm. carotene (*3 rats*)	,,	...	97–143 (average)	All healthy.
0·04 mgm. carotene	107	M.E.+++; N.S.	113–83	No recovery (exceptional result).
Ditto (*3 rats*)	To end of experiment	...	107–156 (average)	All healthy.
0·08 mgm. carotene (*4 rats*)	Ditto	...	114–170 (average)	,,
0·05 gm. dried cabbage (*4 rats*)	,,	...	78–171 (average)	All healthy; more abdominal fat than 0·08 carotene group.
Nil	70	T.A.+++; enteritis; bladder; seminal vesicles	119–136–94	...
,,	80	X.+; T.A.++++; kidneys	120–125–104	Kidneys studded with small septic foci; ureters enormously distended with necrotic septic material; bladder almost unaffected; hyperplasia cardiac end of stomach.
,,	54	X.+; T.A.+	128–135–103	...
,,	84	X.+++; T.A.+++; bladder	95–105–96	Bladder showed great hyperplasia with red sessile projections from mucous membrane.

growth. When cabbage was used as a source of carotene, 0·5 gm. of the dried leaf, equivalent to about 5 gm. of cabbage, always afforded complete protection. In the case of cod-liver oil—a substance containing no carotene but vitamin A in an unknown form—the protection was not complete although 10 mgm. of this substance was used as the supplement. The brand of cod-liver oil was not a particularly good one as regards its vitamin A content, for, as determined by

TABLE III.—*Exp. III: Preliminary Depletion Period. (52 Days on —A Basal Diet prior to adding Supplement.)*

Duration of experiment (total), 161 days.

Nature of supplement after depletion period.	Survival time.	Site and intensity of infection.	Growth response (in gm.).	Remarks.
0·02 mgm. carotene	To end of experiment	None	111–152	...
,,	Ditto	,,	106–159	...
,,	54	T.A.++; X.+; cystitis	...	Marked hæmaturia prior to supplement; no recovery from depletion period.
0·01 mgm. carotene	62	M.E.++; N.S.; X.++	104–85	...
,,	To end of experiment	None	97–124	...
,,	Ditto	,,	101–136	...
0·005 mgm. carotene	84	T.A.++; pyonephrosis; bladder stone	92–71	...
,,	73	Mastoid; enteritis	101–84	Enteritis prior to supplement.
0·01 mgm. carotene + 0·5 gm. dried cabbage (*2 rats*)	To end of experiment	None	99–129 89–111	...
0·5 gm. dried cabbage (*3 rats*)	Ditto	,,	101–147 99–123 84–101	...

the tintometer method of Carr and Price (1925), it only contained 4 units as compared with 9 units or more that would be found in a potent specimen.

The relation of the anti-infective action of carotene to growth requires some consideration, for the crucial biological test of vitamin A has always up to the present depended on its power to influence growth rather than its power to confer resistance to bacterial invasion. It must be pointed out that the experimental periods used in our experiments are much longer than those of other workers. It has been usual to observe the effects of carotene on growth over periods of 28 to 35 days, whereas in our experiments observations were made over periods up to 80 days. It will be noticed that on the whole where no signs of infection were found post-mortem the animals continued to grow to the end of the experiment. Occasionally growth continued, although some infection, generally of a mild type, was found post-mortem. Thus some

infection is compatible with growth. This was especially noticeable in the cod-liver oil group, where the infective foci were slight in degree. It would seem probable from this that cessation of growth and loss of weight under these conditions always indicated an infected state. This, although mainly true, we hesitate to say is always the case, for in a few animals no infective foci

TABLE IV.—*Exp. IV: The Comparative Effects of Carotene, Cod-Liver Oil and Cabbage on Resistance to Infection. No Preliminary Depletion Period.*

Duration of experiment, 74 days.

Nature of supplement.	Survival time.	Site and intensity of infection.	Growth response (in gm.).	Remarks.
0·04 mgm. carotene (*5 rats*)	To end of experiment	*Nil*	56–126 (average) (125% increase)	Perfectly healthy.
0·16 mgm. carotene (*9 rats*)	Ditto	„	59–139 (average) (136% increase)	„ „
0·5 gm. dried cabbage (*5 rats*)	„	„	57–133 (average) (133% increase)	„ „
10 mgm. cod-liver oil	„	Mastoids	54–89	General condition good.
Ditto	„	Mastoid	53–120	„ „
„	„	M.E.+	61–135	„ „
„ (*2 rats*)	„	*Nil*	58–128 (average for C.L.O. group 119% increase)	Perfectly healthy.
Nil	61	X. +; panophthalmitis (L.); T.A.+; bladder; kidney; pelvis	68–125–102	...
„	52	T.A.++	44–59–56	...
„	53	X.+; T.A.+; M.E.++; bladder (with extreme hyperplasia)	56–77–56	...

were found, although loss of weight was evident. It is possible that a septicæmia may have been present in these cases without local foci, but on this point we have no evidence. If the generally accepted test of vitamin A be examined, namely, the property of causing growth resumption for a period of 4 weeks after A depletion, it will be seen that the statement of Euler *et al.* (1928) and Moore (1929), that amounts of the order of 0·005 mgm. of carotene are efficient, may be accepted. It is obvious, however, that the degree of protection against infection brought about by this quantity of carotene is only slight, and that ultimately both loss of weight and death with infective foci result. In other words, the protective dose of carotene is higher than that required to stimulate

TABLE V.—*Summary of Results showing Degree of Anti-Infective Action of Various Doses of Carotene.*

Experiments I and IV (No Depletion Period).

Protective agent.	Severe infection.	Moderate infection.	No infection.
None . . .	5	1	0
0·005 mgm. carotene	1	2	0
0·010 ,, ,,	0	3	0
0·040 ,, ,,	0	0	5
0·160 ,, ,,	0	0	9
0·500 gm. dried cabbage	0	0	5

Experiments II and III (Preliminary Depletion Period).

Protective agent.	Severe infection.	Moderate infection.	No infection.
None . . .	4	0	0
0·005 mgm. carotene	5	1	0
0·010 ,, ,,	0	3	2
0·020 ,, ,,	1	0	5
0·040 ,, ,,	0	1	3
0·080 ,, ,,	0	0	4
0·500 gm. dried cabbage	0	0	7

growth for a short period, the minimal amounts for the two purposes being roughly of the order of 0·02 mgm. (for anti-infective action) and 0·005 mgm. (for growth resumption). Comparative effects of these two quantities of carotene on growth can be seen in the composite curves of Fig. 1. One point of interest in these growth observations, to which attention may be drawn, is that even when no vitamin D was added to the diet, good growth was maintained for a period of 78 days in animals receiving 0·01 mgm. carotene.

We are still not in a position to give any explanation of the mode of action of either vitamin A or carotene in raising the resistance of the body to infection. An earlier discussion, it may be remembered, centred round the question as to which was the first phenomenon produced by lack of vitamin A—the epithelial hyperplasia or infection of the epithelium. We were interested to note that in cases where 0·01 mgm. of carotene was given and the animals developed local septic foci, there was no epithelial hyperplasia obvious to the naked eye in organs other than those infected, and possibly not in these. This may mean either that the hyperplasia had been brought back to normal by the carotene, or that some hyperplasia might still be present, but could only be seen by microscopic examination. Investigations into this and other related problems should now be accelerated, for in carotene we have a substance functioning as vitamin A whose dosage can be accurately controlled and which can be given free from complicating factors.

Euler, Euler and Karrer (1929) have recorded the presence of carotene in the liver after the feeding of carotene to rats. Presumably when storage

occurs the animal is receiving a sufficient supply of carotene for its immediate needs. It is significant in this respect that no carotene was found in the liver by these investigators when the daily dose was below 0·029 mgm., whilst a dose of under 0·005 mgm. was sufficient to restore growth (for the experimental period of 35 days). Our observations on the livers of carotene-fed animals suggest that it is stored, not as carotene, but as vitamin A, as recently indicated by Moore (1929), but we find that the minimal dose required to bring about liver retention is considerably higher than that suggested by Moore—at any rate in rapidly growing animals.

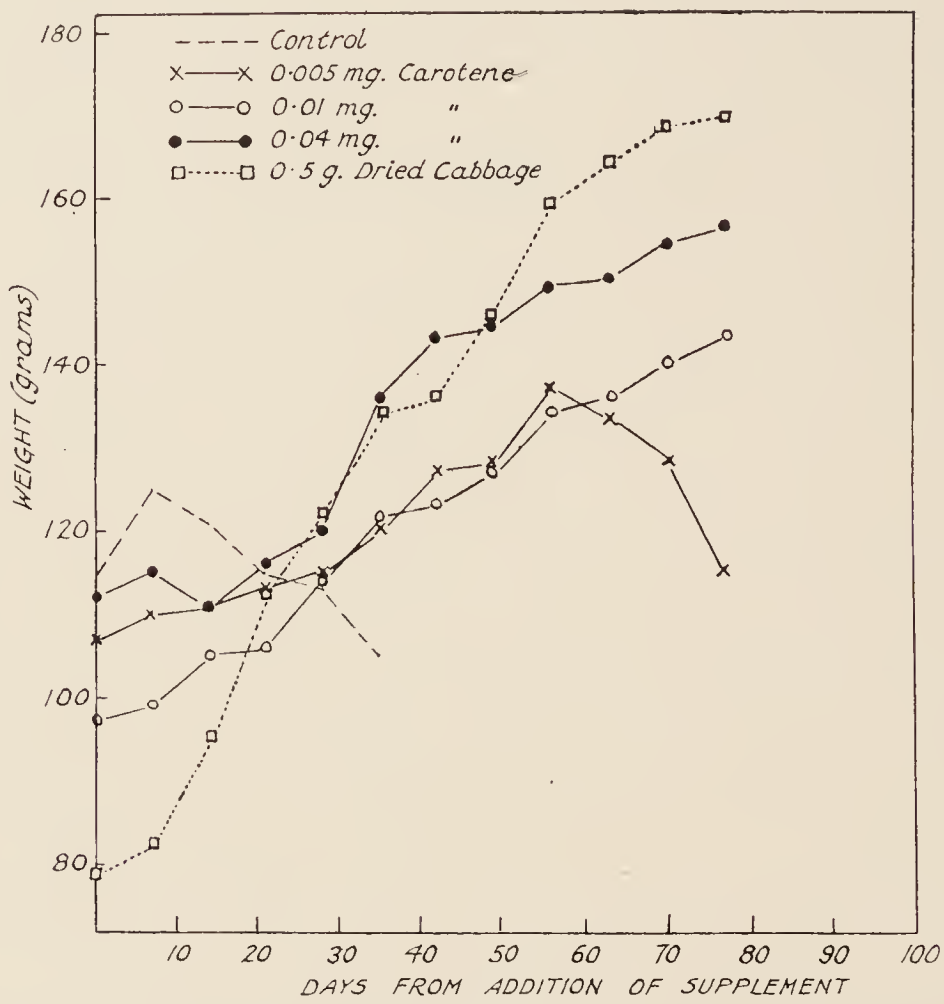

Fig. 1.—The effect of varying quantities of carotene on the growth of young rats after a depletion period (—A diet) of 32 days. Each curve is composite.

In our previous communications we assigned to vitamin A an anti-infective function, though we always realized that there might be other factors associated with it in the green part of the plant or in the liver oil which might play some subsidiary part in this protective function. The fact that carotene itself in sufficient dosage protects the animal completely against this spontaneous infection indicates that the effect is due in both cases to one chemical entity. Since, however, vitamin D, the only vitamin of which we have any quantitative knowledge, protects against rickets in doses as small as 0·0001 mgm.—a much smaller dose than is necessary in the case of carotene when used as an anti-infective agent—we hesitate to ascribe the specific effect definitely to carotene itself, and there is still a slight possibility that the active agent is an impurity—maybe a carotinoid derivative allied to the vitamin A compound as found in liver. The balance of evidence, however, favours the view that carotene is itself the specific substance, and that it is responsible for the vitamin A

properties of green vegetables, carrots and butter and probably egg-yolk. Euler *et al.* (1928) stated that the ether-soluble lipochrome extracted from human blood not only gives the characteristic blue colour with $SbCl_3$ but also the absorption bands of carotene. If this be so, there is a possibility that the liver stores its vitamin A as a highly active leuco form of carotinoid, which may be reconverted to carotene and liberated into the circulation as required.

If carotene has a similar function in man to that in the rat, as seems probable, it should prove valuable both as a prophylactic and therapeutic agent. As a therapeutic agent it should be of special importance for rapid and effective action, for it can be given without the large amount of lipoid material, often badly tolerated, which has to be given in the massive vitamin A therapy with liver-fat preparations.

Incidentally, these results suggest the desirability of a greater use of carrots where vitamin A is indicated because of their relatively large content of carotene as recently emphasized by Moore (1929).

SUMMARY.

(*a*) Tests on a specimen of carotene (melting-point 174°) showed that this substance had the property of conferring complete immunity in growing rats against the development of spontaneous infection.

(*b*) Animals on a diet free from vitamin A and carotene invariably developed septic foci and died. When carotene was given in the food the amount of protection conferred on the animals was generally proportional to the amount of carotene eaten.

(*c*) With the basal diet used in these experiments, carotene in amounts of 0·005 mgm. gave only slight immunity, 0·01 mgm. partial immunity, whereas 0·02 mgm. and greater amounts gave complete or practically complete immunity.

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